REMARKS

Claims 7, 15, 16, 18, 19, 22, and 41-75 are pending, and claims 22 and 46-75 are withdrawn from consideration

The Title of the application is amended to remove the parenthetical phrase objected to by the Examiner.

Claims 7, 15, 18, 19, and 22 are amended to recite "obtained from" in place of "derived."

Regarding the comments in the Office Action concerning the list of references in the specification on pp. 12-13, Applicants note that each of these references has already been cited in an Information Disclosure Statement.

In view of the following remarks, reconsideration is respectfully requested.

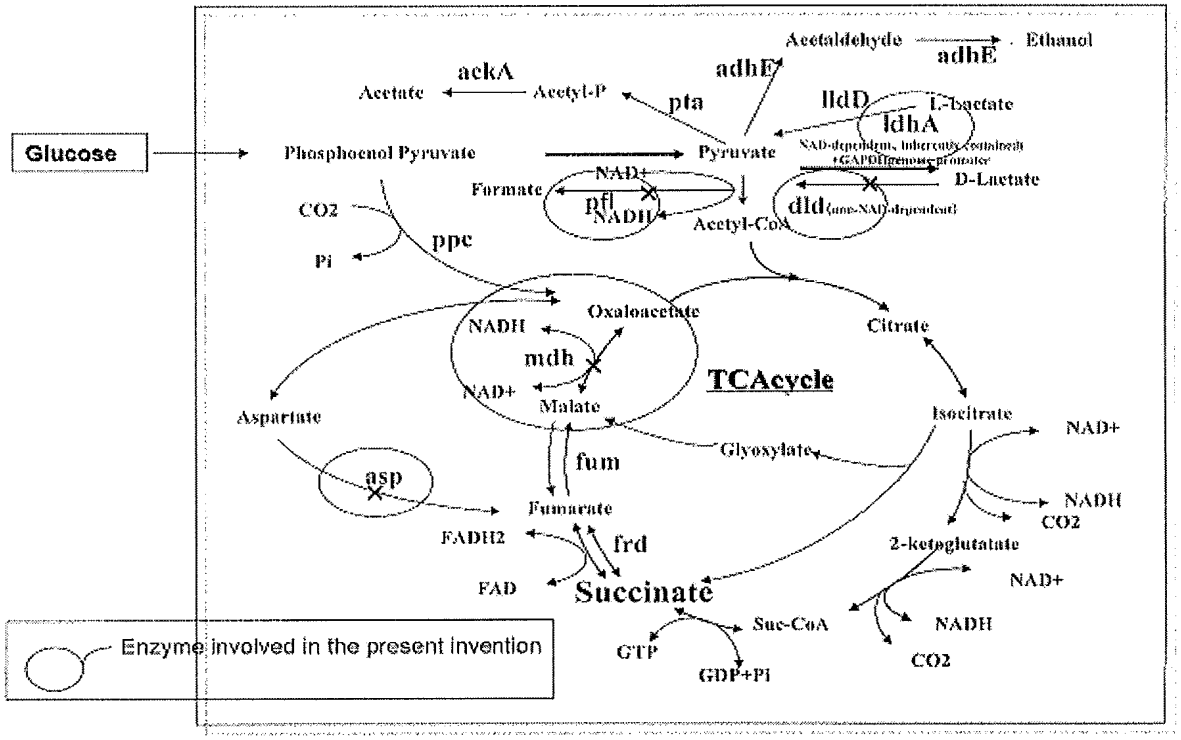
The Claims Are Definite

Claims 18 and 19 were rejected under 35 U.S.C. § 112, second paragraph, as allegedly indefinite. The claims have been amended in view of the Examiner's suggestion, and Applicants respectfully request reconsideration and withdrawal of the rejection.

The Claims Are Nonobvious

Claims 7, 15, 16, 18, 42 and 44 were rejected under 35 U.S.C. § 103(a) as purportedly unpatentable over Zhou et al., (Applied and Environmental Microbiology, Jan. 2003, pp. 399-407) ("Zhou") in view of Yang et al., (Metabolic Engineering, 1999, pp. 141-152) ("Yang") as evidenced by Bunch et al., (Microbiology, 1997, pp. 187-195) ("Bunch") and further in view of Shaw et al., (J. Bacteriology, 1975, pp. 1047-1975). Applicants respectfully traverse the rejection.

The following illustration ("metabolic map") shows biochemical pathways that pertain to the claimed invention (including withdrawn Claim 22), and may prove helpful in understanding the superior and unexpected results provided thereby in comparison with the prior art, in particular the reduced production of undesired products in conjunction with high levels of production of the desired D-lactate.



Zhou discloses production of D-lactic acid by disruption of the pflB, adhE, frdABCD, and ackA genes in *E. coli*, to produce a strain named SZ63. These genes relate to enzymes mediating the following reactions: pflB relates to converting pyruvate to formate; adhE relates to converting pyruvate to ethanol via acetyl-Co A and acetaldehyde; frd ABCD relates to converting succinate to fumarate, or fumarate to succinate; and ackA relates to converting acetyl-P (obtained from pyruvate) to acetate. As noted in the present specification (¶[0010] of the published version), Zhou's *E. coli* produces D-lactic acid with high specificity.

As recognized by the Examiner, Zhou fails to disclose the claimed enhanced activity of NADH-dependent D-lactate dehydrogenase (ldhA) in a microorganism. This enhancement produced superior and unexpected results in the claimed invention. Zhou's SZ63 strain yielded 539 mM in 24 hours (Zhou, p. 403, Table 3), corresponding to 48.5 g/L in 24 hours. In contrast, two strains of the claimed microorganisms produced 115.6 g/L in 48 hours and 113.5 g/L in 30 hours, respectively, a substantial increase. Specification, ¶[0184].

The rejection relies upon Yang for the disclosure of ldhA and upon Bunch for evidence of the ldhA promoter and for a multicopy cloning vector of ldhA. Yang discloses that introducing ldhA gene with a plasmid into an ldhA defective strain leads to metabolic changes including increased production of D-lactic acid. Yang, p. 142, right col. last paragraph, and Table 6B on p. 149. However, Yang's bacteria with increased lhdA also had increased production of pyruvate, formate, hydrogen, ethanol, and acetate. Yang, p. 149, Table 6A. Such increased levels of these products, other than the desired D-lactic acid, are not preferred from the industrial viewpoint, since it would be necessary to remove those products. Specification, ¶[0006].

In contrast, the present inventors were able to achieve unexpected and surprising reductions in pyruvate by inactivating pfl while enhancing ldhA. Example 7, ¶[0149], Table 7. Disruption of pfl alone (MG1655Δpfl) resulted in the same level of pyruvic acid as the control (MG1655), however pfl deletion in combination with enhanced ldhA (MG1655Δpfl / pGlyldhA) desirably and surprisingly reduced pyruvic acid. Id. This result is in surprising contrast to Yang, where increased ldhA *increased* levels of pyruvate. Yang, p. 149, Table 6A. The rejection lacks support

for a contention that one of ordinary skill in the art would expect a reduction in pyruvate production on disruption of pfl: in fact, given the role of pfl, the opposite could be expected.

Shaw is relied upon for disclosure of strains lacking L- and D-lactate dehydrogenases. However, data of the present application surprisingly demonstrate that the claimed gene disruption of dld desirably reduced the accumulation of undesired products when ldhA was enhanced. Specification, ¶[0178], (Example 17) and Table 23. In this Example 17, the accumulation of various products, D-lactic acid, pyruvic acid, formic acid and acetic acid, are shown of each of various strains. Id. In comparing strain (B) (in which dld is disrupted) with strain (E) (in which dld is disrupted and ldhA gene enhanced), strain (E) had both pyruvate and acetate are surprisingly and desirably reduced compared to strain (B). Id. Again, the rejection lacks support for a contention that such a result would be expected.

While the rejection alleges that it would be obvious to try various combinations of genetic manipulations in order to increase production of D-lactate, the reduced production of undesired products that results from the claimed invention is entirely unexpected and nonobvious. In fact, the evidence of record suggests that an increase in undesired products would be expected with enhanced ldhA. The results of Yang were cited above (increased production of pyruvate, formate, hydrogen, ethanol, acetate, seen in Table 6A). The reference further recited that the positive amplification factors (or deviation indices) for all three branches implies that an increase in the LDH activity not only increases its own flux and the common flux, but also increases the flux of the competing branch, i.e. unwanted reaction products. Yang, p. 148, second column, last paragraph *et seq.*

Furthermore, Applicants respectfully submit that, given the large number of enzymes in the relevant biochemical pathways, together with the possibility for genetic modification of nearly any number of them simultaneously, the resulting number of combinations is so large that it would be practically impossible to try all such combinations. When no direction as to which of many possible choices is likely to be successful, an invention would not have been obvious to try. Bayer Schering Pharma AG v. Barr Labs. Inc., 91 USPQ2d 1569, 1573 (Fed. Cir. 2009). Accordingly, no *prima facie* case of obviousness exists.

The highly unpredictable field of art renders the obviousness rejection even less tenable. The relevant biochemical pathways involve numerous enzymes, a complex interplay among their various reaction products, and a requirement for at least some unperturbed functionality for survival of the microorganisms. Yang refers to the "highly complicated regulatory factors" of the pyruvate formate lyase branch. Yang, p. 150, first column, first paragraph. Furthermore, in Bunch, it was reported that pfl IdhA double mutants cannot grow anaerobically on any sugar or sugar alcohol even when supplemented with acetate, in contrast to single IdhA mutants, which show no anaerobic growth defect. Bunch, p. 191, right column, last paragraph. Such difficulties would severely hamper the extensive trials of various combinations of genetic manipulations proposed in the rejection.

Even if one of ordinary skill in the art were to combine the references as applied in the rejection to arrive at the claimed subject matter, the record fails to support a contention that the resulting high level of productivity of D-lactate together with low production of other compounds would be expected. Applicants respectfully submit that the record as a whole indicates that the claimed invention in nonobvious.

Claims 41, 43 and 45 were rejected under 35 U.S.C. § 103(a) as purportedly unpatentable over Zhou in view of Yang as evidenced by Bunch and further in view of Shaw and yet further in view of Courtright et al., (J. Bacteriology, 1970, pp. 722-728) ("Courtwright"). Applicants respectfully traverse the rejection.

Courtwright is relied upon for *E. coli* mutants devoid of malate dehydrogenase activity. Applicants respectfully submit that Courtwright fails to cure the above-noted deficiencies of Zhou, Yang, Bunch, and Shaw. Additionally, it is respectfully submitted that Claims 41, 43, and 45 are further patentable for the below reasons.

The primary reference Zhou discloses deactivation of frd for reduced succinate, whereas the invention of Claims 41, 43, and 45 has inactivated or decreased mdh and does not recite modification of frd.

As described in the present specification, a mutant obtained by disrupting frd in the strain MG1655ΔpflΔdld (referred to "MG1655ΔpflΔdldΔfrd") produces D-lactic acid in an amount of 71 g/L in 32 hours, and a strain according to the invention MG1655ΔpflΔdldΔmdh produces D-lactic acid in an amount of 89 g/L in 32 hours. Specification, ¶¶[0200] - [0201]. Accordingly, the amount of D-lactic acid produced by the claimed microrganism should be significantly higher than that by the mutant corresponding to mutant of Zhou. Neither of these strains accumulated succinic acid.

Referring to the present application, FIGS. 2 and 3, and Example 24 (¶¶ [0199] - [0201]), it was found by the present inventors that, as compared with the control MG1655ΔpflΔdld, strains corresponding to that of Zhou (namely MG1655ΔpflΔdldΔfrd) would appear to inhibit the accumulation of D-lactic acid, while inhibiting the accumulation of succinic acid. On the other hand, the mutant of the

invention MG1655ΔpflΔdldΔmdh enhances the accumulation of D-lactic acid, while inhibiting the accumulation of succinic acid.

As seen in the metabolic map previously provided in this paper, one of ordinary skill in the art would expect mdh to operate similarly to frd and fum with regard to influencing the accumulation of succinate. However, the present inventors finds that mdh performs in the different way from frd and fum, that is, the inactivation either of frd or fum gene leads to inhibition of the accumulation of succinic acid as well as D-lactic acid (Comparative Example 2 and FIGS. 2 and 3). On the other hand, the inactivation of mdh gene leads inhibition of the accumulation of succinic acid and accumulation of D-lactic acid.

Further, one would expect from the metabolic map that when the ppc gene (phosphoenol pyruvate to oxaloacetate) is inactivated, there would be an increase in the amount of pyruvate, so that D-lactic acid should be accumulated. However, Comparative Example 1 and FIGs. 2 and 3 in the present application shows the opposite trend, that is, the inactivation of ppc gene leads to inhibition of accumulation of D-lactic acid as well as succinic acid.

In the other words, the inactivation of mdh should not be expected to result in accumulation of D-lactic acid, based on the results with either of frd, fum or ppc.

Courtright discloses that mdh gene is inactivated in a strain derived from E. coli K12, so that succinic acid (succinate) is not produced (p. 725, Table 3). However, the present inventors find the following (¶¶ [0200] - [0201]): accumulation of succinate by a strain modified to disrupt pfl and dld genes is inhibited by inactivating either of mdh, ppc or frd gene; and that the highest productivity of D-lactic acid is obtained by inactivating mdh gene. It is highly desirable that D-lactic acid is produced at the highest level of the productivity while bringing the production

of succinate close to zero, not merely that succinic acid is produced at a lower level of the productivity. Specification, ¶[0006].

Courtright along with the other references as applied fail to contemplate the superior and unexpected results obtained by the claimed invention.

Claims 18 and 19 were rejected under 35 U.S.C. § 103(a) as purportedly unpatentable over Zhou in view of Yang as evidenced by Bunch and further in view of Shaw and yet further in view of Maier et al., (U.S. Patent Application No. 10/620,487, filed July 16, 2003) ("Maier"). Applicants respectfully traverse the rejection.

Maier is relied upon for disclosure of a GADPH promoter. Applicants respectfully submit that Maier fails to cure the above-noted deficiencies of Zhou, Yang, Bunch, and Shaw. Additionally, it is respectfully submitted that Claims 18 and 19 are further patentable for the below reasons.

Two strategies may be considered for expression of ldhA linked to a GAPDH promoter: one is by use of multicopy plasmid, and the other is insertion into the genome. It is submitted that one of ordinary skill in the art would expect that the use of multicopy plasmid should realize higher expression level than that by insertion into a genome, by virtue of having multiple copies available for transcription and subsequent translation, and therefore would employ this method rather than the claimed genomic integration. *See also* Specification, ¶[0020].

However, the present inventors surprisingly and unexpected found that the productivity of D-lactic acid in E. coli by improving the expression level of ldhA gene by GAPDH promoter, and further that the productivity of D-lactic acid with GAPDH promoter introduced by insertion into a genome is much higher by use of plasmid.

Specification, ¶[0048]. Neither Maier nor the other references as applied teach or suggest that this might be the case.

Conclusion

For the foregoing reasons, allowance of the application is respectfully requested. If there are any questions concerning this response, Applicant's undersigned representative can be reached at the number below.

The Director is hereby authorized to charge any appropriate fees under 37 C.F.R. §§ 1.16, 1.17 and 1.20(d) and 1.21 that may be required by this paper, and to credit any overpayment, to Deposit Account No. 02-4800.

Respectfully submitted,

BUCHANAN INGERSOLL & ROONEY PC

Date: December 23, 2009 By: _____

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